Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/IL04/001188

International filing date: 30 December 2004 (30.12.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/533,230
Filing date: 31 December 2003 (31.12.2003)

Date of receipt at the International Bureau: 08 February 2005 (08.02.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

PCT/IL2004/001188

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APPLICATION NUMBER: ***60/533,230***
FILING DATE: ***December 31, 2003***

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13281 U.S.PTO
123103

22581 U.S.PTO
60/533230
123103

U.S. PATENT AND TRADEMARK OFFICE
PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 C.F.R. §1.53(b)(2)

Atty. Docket: GARTI2

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[ ] Additional inventors are being named on separately numbered sheets attached hereto

TITLE OF THE INVENTION (280 characters max)

STABLE ILL-DEFINED CUBIC NANOSIZED PARTICLES IN A TERNARY AQUEOUS PHASE

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ENCLOSED APPLICATION PARTS (check all that apply)

[X] Specification Number of Pages 10 [X] Applicant claims small entity status. See 37 C.F.R. §1.27

[X] Drawing(s) Number of Sheets 6 [ ] Other (specify) ______

METHOD OF PAYMENT (check one)

[X] Credit Card Payment Form PTO-2038 is enclosed to cover the Provisional filing fee of

[ ]$160 large entity [X] $80 small entity

[X] The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number 02-4035

The invention was made by an agency of the United Stated Government or under a contract with an agency of the United States Government.

[X] No [ ] Yes, the name of the U.S. Government agency and the Government contract number are:

______

Respectfully submitted,

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Date: December 31, 2003

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STABLE ILL-DEFINED CUBIC NANOSIZED PARTICLES IN A TERNARY AQUEOUS PHASE

FIELD OF THE INVENTION

The present invention relates to a stable semi-ordered ill-defined single phase with cubic symmetry in a ternary aqueous system.

BACKGROUND OF THE INVENTION

Micelle microemulsions (water/oil, bicontinuous, oil/water) and lyotropic liquid crystals are some of the well-known and well-studied phases that amphiphilic entities adopt when they are in aqueous vicinity. Lyotropic liquid crystalline mesophases (lamellar, hexagonal reverse hexagonal, cubic etc.) are well characterized and employed in numerous applications. Within the large family of liquid crystalline phases, the bicontinuous cubic phase has attracted much attention since its first description (Luzzati, V., Tardieu, A., Gulik-Kryzwicki, T,. Rivas, E., Reiss-Husson, F. (1968) *Nature* 220, 485). It is well-defined and characterized by spectroscopic and spectophotometric measurements. Its small angle X-ray scattering and ^{13}C NMR spectroscopy are given in Minoru, N., Atsuhiko, S., Hideki, M., Tetsurou, H. (2001) *Langmuir* 17, 3917. A review by one of the inventors of the present invention, titled "*Bicontinuous Liquid Crystalline Mesophases-solubilization Reactivity and Interfacial Reactions*" recently sent to publication, summarizes its vast use in research and furthermore, its potential use as a substitute for solubilizing hydrophilic and hydrophobic materials for sustained and controlled release. The latter use of the cubic phase is attributed to its extremely large surface area, well organized microstructure. However, like all liquid crystalline phases, the semisolid or gel-like macrostructure can not be used as is for solubilizing hydrophilic and hydrophobic material because it is glassy and non-

dispersible and therefore the cubic phase should be diluted or dispersed in an appropriate aqueous system and solvent. Dilution and dispersion were successfully done where they involve use of additional specific (mostly polymer) hydrophilic surfactant and co-solvent like alcohol or some other high shear force. Dilution should be done cautiously, since it may result in disruption of the microscopic "order" and at high dilution ratios may completely distort microscopic structure leading to loss of their unique character.

SUMMARY OF THE INVENTION

The present invention is based on the fact that a ternary system comprising water, alcohol and fatty acid or an ester thereof, may form spontaneously a stable, non-viscous and clear nanosized structures having cubic-like nanosized symmetry. The ternary system being a single phase is created in well-defined concentrations of the three components of the system. Outside the boundaries of these relative concentrations, other known single phase or biphasic solutions prevail (non-continuous, two-phase, etc.). The spontaneously formed ternary system is capable of being diluted or dispersed in a water/polymer at room temperature and/or 9000 rpm to form dispersed cubic-like nanosized particles.

Thus according to a first embodiment the present invention is directed to a ternary system comprising:

(i) 35 to 75% water;

(ii) 0 to 30% alcohol; and

(iii) 15 to 65% fatty acid or an ester thereof.

The alcohol is a C_1-C_8 alcohol or a polyalcohol. Preferably the alcohol is ethanol, propanol or butanol or polyethylene glycol. The fatty acid is a C_2-C_{22} saturated or unsaturated fatty acid wherein the unsaturated fatty acid may contain one or more double bonds. The fatty acid ester may be with a regular alcohol or a polyalcohol such as glycerol, sorbitol, propylene glycol. Preferably it is glycerol esters of fatty acids. Most preferably it is glycerol monooleate or a mixture of

monooleate and monostearate or any partially hydrogenated monoglycerol of vegetable oils.

The present invention according to a second embodiment is further directed to ternary system comprising water, alcohol and fatty acid or an ester thereof, forming spontaneously a stable, non-viscous and clear nanosized structures having cubic-like nanosized symmetry for use in solubilizing hydrophilic or hydrophobic substances in aqueous phase for use in solubilizing hydrophilic, hydrophobic, or non-water or non-oil soluble substances. The spontaneously formed ternary system is capable of being diluted or dispersed in a water/polymer at room temperature and/or 200-20000 preferably 9000 rpm to form dispersed cubic-like nanosized particles which are used for solubilizing hydrophilic, hydrophobic or non-water or non-oil soluble substances. Such substances may be enzymes vitamins, pharmaceuticals, peptides or food supplements.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to understand the invention and to see how it may be carried out in practice, a preferred embodiment will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 is phase diagram of a ternary aqueous phase system comprising water, ethanol and glycerol monooleate; **(A)** of the prior art showing the various ordered and semi-ordered structures as a function of the relative concentrations of each of the three components comprising the system; **(B)** of the present invention where in addition to the known ordered phases from the prior art, a semi-ordered heein defined as Q_L phase is present.

Fig. **2** demonstrates a comparison of Small Angle X-ray diffractogram of **(A)** cubosomes (disperesed cubic phase) and of **(B)** the "semi-ordered" Q_L phase (cubic phase) of the present invention

Fig. **3** demonstrates that the "semi-ordered" Q_L phase of the present invention is comprised of single particles having a very narrow distribution of particle size.

Fig. 4 is a cryo-TEM image of large, ordered cubosomes made from the semi-ordered Q_L phase upon dilution (homogenization not required) in a water/polymer system.

Fig. **5** is a cryo-TEM image of the pure "semi-ordered" oil-like Q_L phase without dilution in a water/polymer solution.

DETAILED DESCRIPTION OF THE INVENTION

A well known and characterized cubic phase is formed (among other phases) by mixing glycerol monooleate with water. Upon addition of a diluting co-emulsifier or co-solvent such as an alcohol (mono- or poly-alcohol) one obtains a ternary phase diagram **10 (Fig. 1A)** exhibiting several phase regions. As displayed in the ternary phase diagram **10** several phases exist within the dilution boundaries. An isotropic phase at **20**, a lamellar phase at **30** and two well-charecterized cubic phases at **40** (diamond bicontinuous P_{n3m}) and **50** (gyroid bicontinuous I_{a3d}). The formed cubic phase is a viscous clear bulk. The main bicontinuous cubic phases may be characterized by dispersing the formed cubic phase, for example by addition of a polymer/water (excess water) forming cubosomes. The formed cubosomes are characterized by their small angle X-ray, particle size and their characteristic cryo-Transmission Electron Microscopy (cryo-TEM) images (data not shown). As mentioned, the formed cubic phase may be dispersed, maintaining its mono ordered structure by mechanical or ultrasonic energy together with addition of a water/polymer diluting solution (excess of water) or by dilution with an appropriate system to form cubosomes. The polymer may be a synthetic polymer, natural occurring polymer such as a specific protein or hydrocolloid. The latter method for forming cubosomes is preferable since the exerted mechanical force may degrade the cubic structure. An appropriate system for dilution is a water/polymeric solution, where a polymer of an appropriate length and molecular weight should be used. Upon the addition of the cubic phase to the water/polymer (excess of water) system, cubosomes are formed which can be further used.

Turning to **Fig. 1B** there is described a phase diagram ternary aqueous system of the present invention **60** comprising of water, glycerol monooleate (GMO) and ethanol. As mentioned above, GMO forms lyotropic liquid crystals when mixed with a polar solvent such as water. Addition of ethanol in small concentration to a water/GMO system reduces viscosity, however, in case large amounts of ethanol are added the ordered structure is distorted and the liquid may separate into two phases. As demonstrated in the ternary phase diagrams **10** (Fig. 1A) and **60** (Fig. 1B), the cubic phase exists only when the amount of the ethanol is low (up to 10% in 1A and even a smaller amount in 1B). The microscopic structure of the formed liquid crystal transforms as a function of the temperature. At 25°C it may exist as a lamellar or cubic phase while at 80°C it is in the hexagonal phase. Along the GMO-water axis **70**, with no ethanol, a lamellar (L_α) phase exists in case the water concentration is up to 17% (**80**). As the concentration of water is increased, i.e. at 25% a cubic phase predominates at **90**. Upon addition of ethanol, the lamellar phase predominates (**80**) although also at certain relative concentrations of the three components a cubic phase also exists. Each of these lamellar and cubic phases are well defined and characterized by their X-ray diffraction (after the appropriate dispersion).

At a water concentration in the range of 35% to 75% and at an ethanol concentration of 0% to 30% a unique new "semi-ordered" stable single phase system exists **100**. Outside the defined "window" of relative concentrations of the three components there exists a two-phase system at **110**. The stable single phase, hereinafter termed Q_L is unique and different not only in its macroscopic behavior where it appears as a clear oil-like solution as opposed to clear, however, viscous gel-like liquid crystal appearance but also in its microscopic characters. The Small Angle X-ray (SAXS) diffraction of the clear phase is uniquely different than that of the cubosomes (which are merely a dispersion of a cubic phase) as demonstrated in **Fig. 2** when comparing the diffraction of the pure cubic phase (**2A**) to that of the Q_L phase (**2B**) of the present invention. The semi-ordered Q_L phase comprises particles having a very narrow size distribution as demonstrated in **Fig. 3**. The Q_L phase is

stable for a long period of time at 15 to 33°C (e.g. for a year). Lowering the temperature (to about 7°C) causes a change evident by the formation of turbidity. However, the change is reversible and raising the temperature yields once again the clear oil-like Q_L phase. The dilution of the oil-like Q_L phase in a water/polymer system exhibits a solution whose Cryo-TEM image is similar to that of a cubosome (**Fig. 4**). Different areas **120, 130, 140** and **150** of the same phase are displayed in the Fig. 4. **Fig. 5** further displays the cryo-TEM image of the pure non-diluted oil-like Q_L phase demonstrating its resemblance to a dispersed cubic phase, however, the big benefit is the fact it can be used as is without dilution.

The invention, therefore, concerns a ternary system comprising water, alcohol and fatty acid or an ester thereof, forming spontaneously a stable, non-viscous and clear nanosized structures having cubic-like nanosized symmetry. Macroscopically, the system is an oil-like phase. The alcohol used as a diluting solvent for the water/fatty acid or its ester may be a C_1-C_8 alcohol or a polyalcohol. Preferably it is ethanol, propanol or butanol or polyethylene glycol. In a preferred embodiment where the alcohol is ethanol and the fatty acid is in the form of an ester, glycerol monooleate, the relative concentrations of each component yielding the semi-ordered phase is 35% to 75% water an 0% to 30% ethanol. Although not necessary, the spontaneously formed ternary system is capable of being diluted or dispersed in an excess of a water/polymer system at room temperature and/or 200-20000 preferably 9000 rpm to form dispersed cubic-like nanosized particles. The polymer may be a high molecular weight amphiphilic synthetic or naturally occurring polymer or a mixture thereof. Non limiting examples of a synthetic polymer are PEG-100, PEG-60. A naturally occurring polymer may be β-casein. Compared to the cubosomes which are formed by dispersing cubic phase particles, the "semi-ordered" phase of the present invention is thermodynamically very stable. Thus the ternary system of the present invention has three major advantages over the cubosomes. It does not have to be diluted prior to its use since it is an oil-like phase as opposed to the gel-like phase of the liquid crystalline cubic phase. In

case dilution is desired, it may be done at room temperature with no need of any shear force. Furthermore, it is stable for longer periods of time, e.g. a year.

Well ordered liquid crystals have many applications all utilizing their relative structured character and the very large surface area they posses. The fact that the Q_L oil-like semi-ordered phase of the present invention may be used as is with no need to further dilute it is a big advantage for its use as a solubilizing medium. It thus may be used as is for solubilizing hydrophilic and hydrophobic compounds such as enzymes, vitamins, food supplements, pharmaceuticals or dyes, antioxidants, perfumes or peptides. Lycopene, a hydrophobic food supplement and ascorbic acid, a hydrophilic vitamin were successfully solubilized in an aqueous phase comprising of the Q_L semi-ordered phase having a water/GMO/ethanol relative concentration of 60%/40%/10%.

Experimental

<u>Example 1: Formation of a Q_L "semi-ordered" phase</u>

2gr of GMO were melted by heating to about 50°C. In a separate vessel, 2gr of GMO and 0.5gr of ethanol were placed. The vessel was closed and its contents were mixed well with vortex for several minutes. The vessel was placed in a bath at 45°C. To the vessel was added 2.5gr water. Following the addition of the water the mixture appeared to be white. The mixture was stirred and allowed to stand at room temperature, where after several hours all the foam disappeared and the sample became transparent.

<u>Example 2: Solubilization of lycopene in a Q_L semi-ordered phase</u>

2gr of GMO were melted by heating to about 50°C. In a separate vessel, 2gr of GMO and lycopene (0.0085gr) were placed. The vessel was closed and its contents were mixed well with vortex until all the lycopene dissolved. 0.5gr ethanol was added and the combination further mixed. The vessel was placed in a bath at a temperature of 45°C. 2.5gr water were added. Following the addition of water the mixture appeared white. The contents were further mixed and left to stand at room

temperature where after several hours all the foam disappeared and the sample became transparent.

Example 3: Solubilization of ascorbic acid in a Q_L semi-ordered phase

2gr of GMO were melted by heating to about 50°C. In a separate vessel, 2gr of GMO and 0.5gr of ethanol were placed. The vessel was closed and its contents were mixed well with vortex. The vessel was placed in a bath at 45°C. To the vessel was added 2.5gr water containing ascorbic acid (0.0166gr). Following the addition of the water the mixture appeared to be white. The mixture was stirred and allowed to stand at room temperature, where after several hours all the foam disappeared and the sample became transparent.

CLAIMS:

1. A ternary system comprising water, alcohol and fatty acid or an ester thereof, forming spontaneously a stable, non-viscous and clear nanosized structures having cubic-like nanosized symmetry.

2. A ternary system according to claim 1 wherein said formed non-viscous and clear nanosized structures having cubic-like nanosized symmetry is capable of being diluted or dispersed in a water/polymer at room temperature and/or 200-20000 preferably 9000 rpm to form dispersed cubic-like nanosized particles.

3. A ternary system according to claim 1 comprising:

(i) 35 to 75% water;

(ii) 0 to 30% alcohol; and

(iii) 15 to 65% a fatty acid or an ester thereof.

4. A ternary system according to claim 3 wherein said alcohol may be a C_1-C_8 alcohol or polyalcohol.

5. A ternary system according to claim 4 wherein said alcohol is ethanol, propanol, butanol, pentanol, hexanol, heptanol or octanol.

6. A ternary system according to claim 4 wherein said polyalcohol is polyethylene glycol, propylene glycol, glycerol, sorbitol, manitol or xylitol.

7. A ternary system according to claim 3 wherein said fatty acid is C_2-C_{22} saturated or unsaturated.

8. A ternary system according to claim 7 wherein said unsaturated fatty acid has one or more double bonds.

9. A ternary system according to claim 3 comprising a fatty acid ester.

10. A ternary system according to claim 9 wherein said fatty acid ester is glycerol ester preferably glycerol monooleate.

11. A ternary system according to claim 2, where said polymer is selected from the group consisting of high molecular weight amphiphilc synthetic or naturally occurring polymer.

12. A ternary system according to claim 11 wherein said natural occurring polymer is β-casein.

13. A ternary system comprising 35 to 75% water, 15 to 45% glycerol monooleate and 0 to 30% ethanol.

14. A ternary system comprising water, alcohol and fatty acid or an ester thereof, forming spontaneously a stable, non-viscous and clear nanosized structures having cubic-like nanosized symmetry for use in solubilizing hydrophilic or hydrophobic substances in aqueous phase.

15. A ternary system according to claim 14 wherein said formed non-viscous and clear nanosized structures having cubic-like nanosized symmetry is capable of being diluted or dispersed in a water/polymer in room temperature and/or 200-20000 rpm to form dispersed cubic-like nanosized particles.

16. A stable ternary system according to claim 14 comprising

(i) 35 to 75% water;

(ii) 0 to 30% alcohol; and

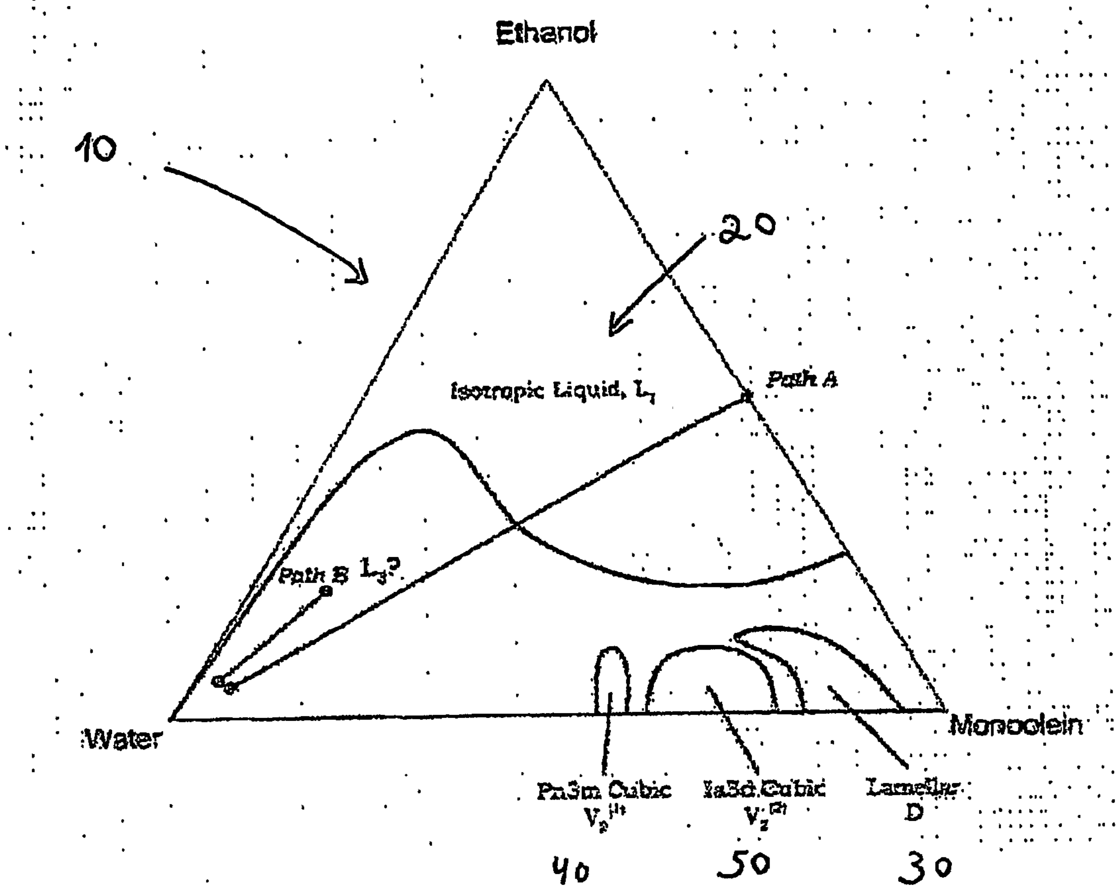
(iii) 15 to 65% fatty acid or an ester thereof.

17. A stable ternary system of claims 14 or 15, wherein said solubilized substnaces are chosen from the group comprising of enzymes, vitamins, pharmaceuticals, peptides, or food supplements.

18. A stable ternary system of claims 14 or 15, wherein said hydrophobic substance is lycopene.

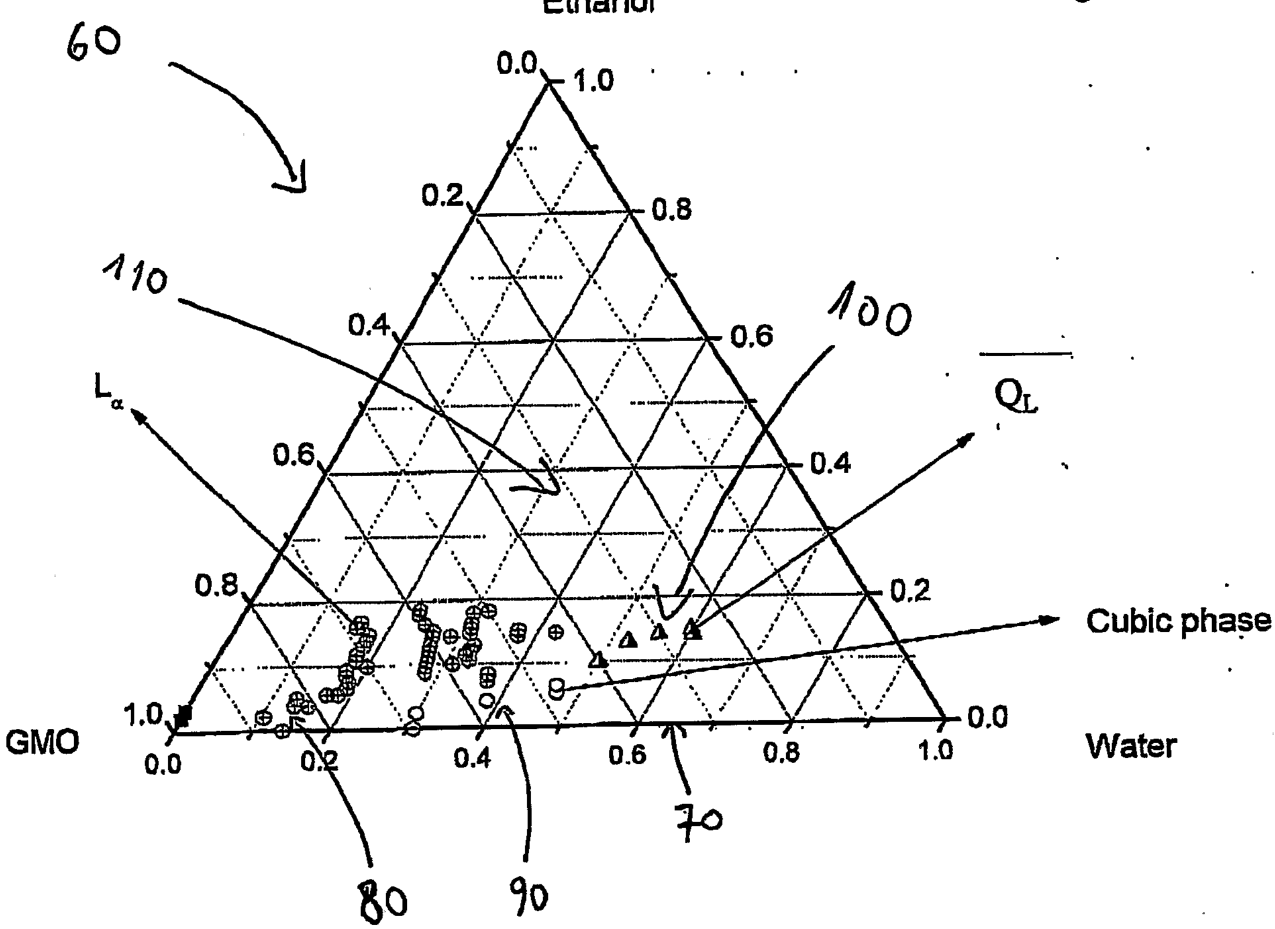
19. A stable ternary system of claims 14 or 15, wherein said hydrophilic substance is ascorbic acid.

Figure 1A



Ethanol
10
20
Isotropic Liquid, L_1
Path A
Path B L_3?
Water
Monoolein
Pn3m Cubic
$V_2^{(I)}$
Ia3d Cubic
$V_2^{(II)}$
Lamellar
D
40
50
30

Fig. 1B



60
Ethanol
0.0
1.0
0.2
0.8
110
0.4
0.6
100
0.6
0.4
Lα
QL
0.8
0.2
Cubic phase
1.0
0.0
GMO
0.0
0.2
0.4
0.6
0.8
1.0
Water
70
80
90

Figure 2: (A) A typical cryo-TEM image of the L4 phase. Semi-ordered regions are the only structures found. (B, C) SAXS scattering curves of the cubic phase found at 75/25 MO/water (B) and of the L4 phase (C).

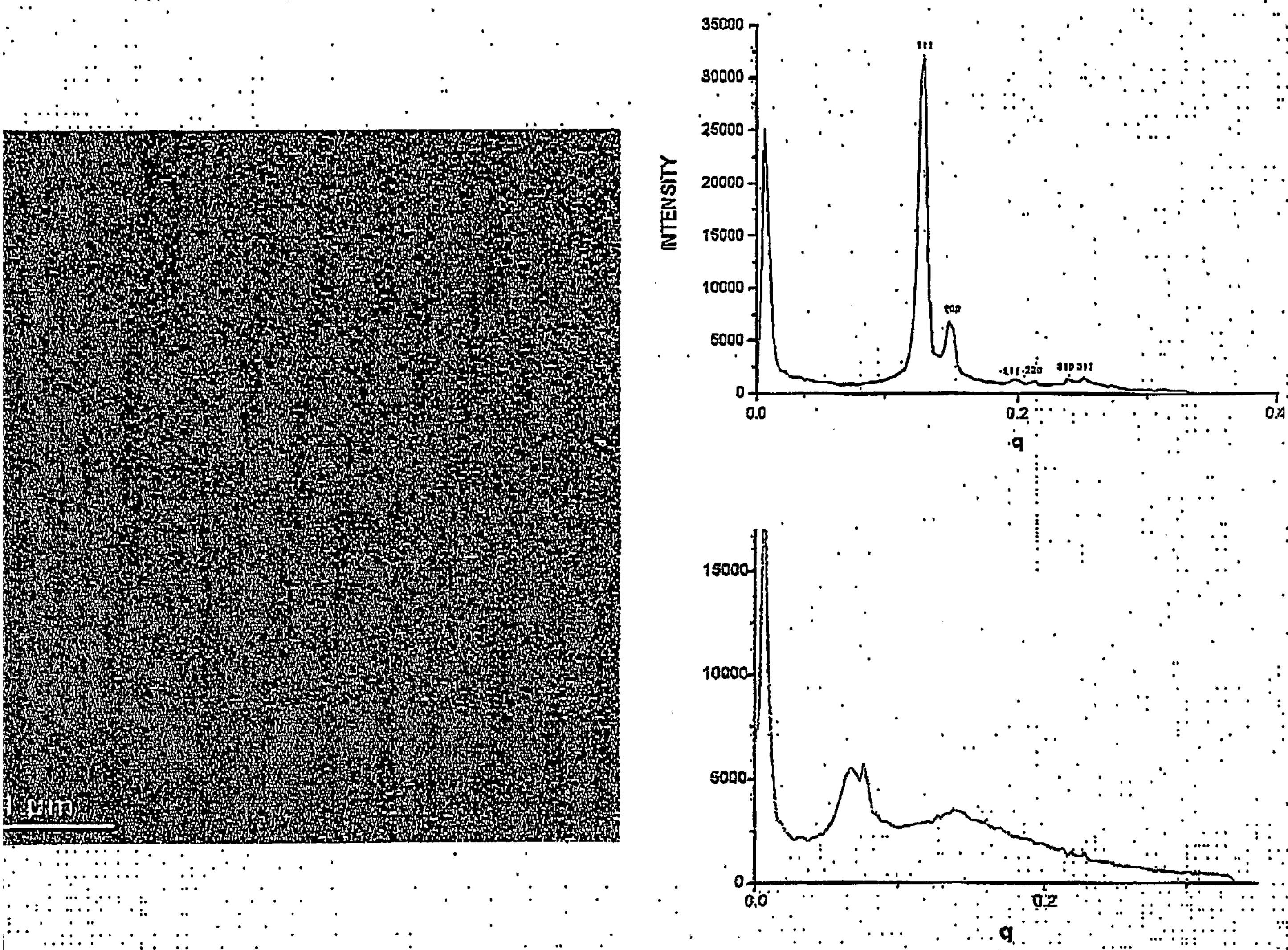


Fig. 3

5b
File data from Live size Record 3
Zetasizer 3000HS

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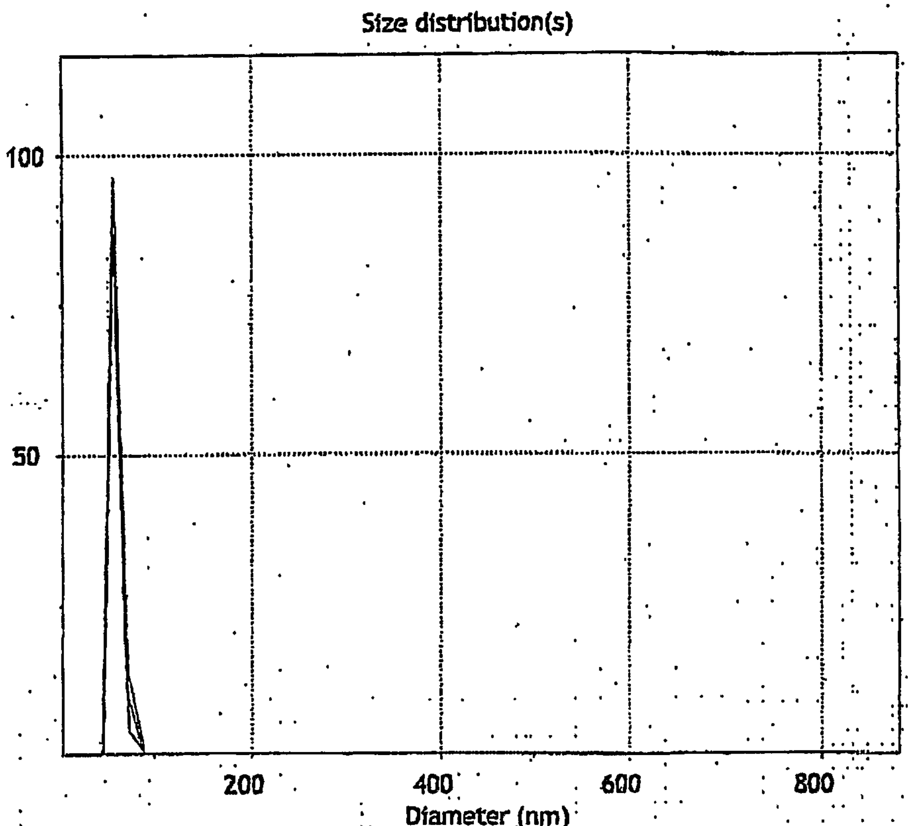
2	90.0	116.0	51.6	0.385	0.000597	09:21:53
3	90.0	116.5	51.1	0.379	0.000857	09:26:29
Average		115.7	51.0	0.385		
+/-		0.9	0.6	0.006		



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2	90.0	116.0	51.6	0.385	Pass	5.97e-004	Auto:CONTIN
3	90.0	116.5	51.1	0.379	Check Duration	8.57e-004	Auto:CONTIN

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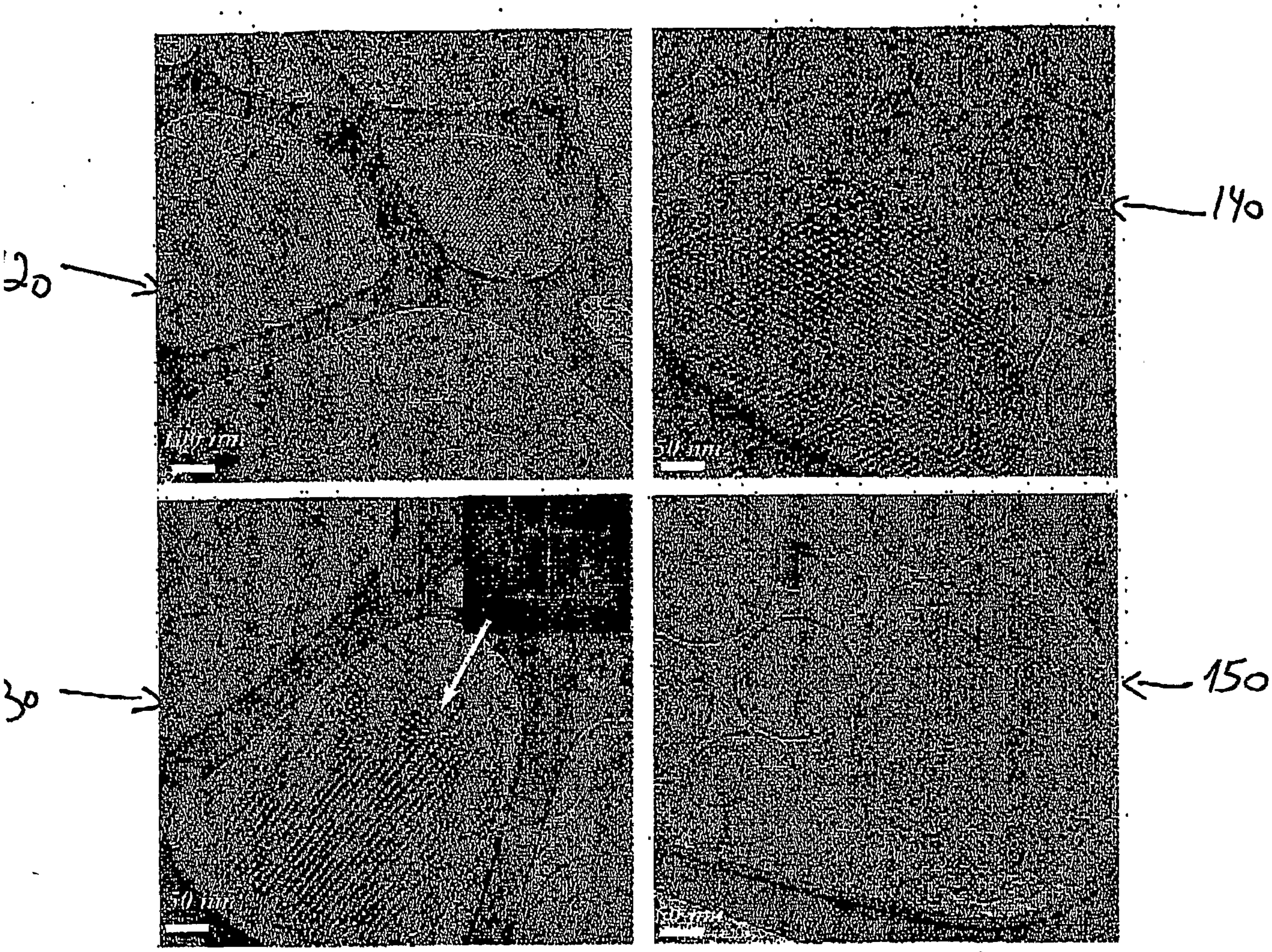

Fig. 4

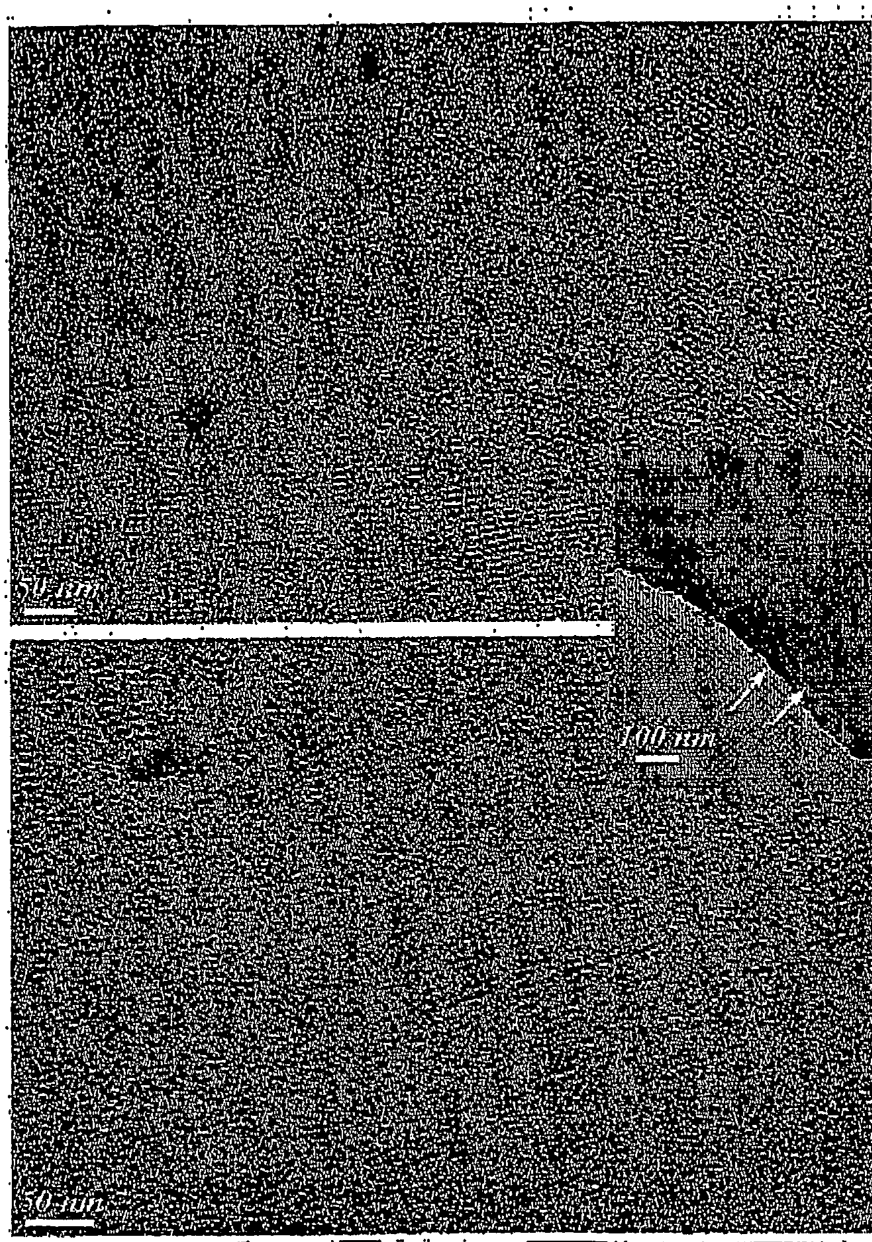

Fig. 5